Image 1642

PTO/SB/21 (08-03)

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	09/381,497
Filing Date	February 17, 2000
First Named Inventor	FITZGERALD, David J.
Art Unit	1642
Examiner Name	Helms, Larry R.
Attorney Docket Number	015280-317100US

Total Number of Pages in This Submission

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☒ Amendment/Reply- **"Supplemental Response"**
  - ☐ After Final
- ☒ Affidavits/declaration(s)- **"Declaration of Dr. David J Fitzgerald Under 37 CFR 1.132"**
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Response to Missing Parts/ Incomplete Application
  - ☐ Response to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s)
- ☐ After Allowance Communication to Group
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to Group (**Appeal Notice, Brief, Reply Brief**)
- ☐ Proprietary Information
- ☐ Status Letter
- ☒ Other Enclosure(s) *(please identify below)*:

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Remarks The Commissioner is authorized to charge any additional fees to Deposit Account 20-1430.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual	Townsend and Townsend and Crew LLP Jean M. Lockyer, Ph.D. Reg. No. 44,879
Signature	
Date	March 11, 2004

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below.

Typed or printed name	Malinda C. Dagit		
Signature	Malinda C Dagit	Date	11 March 2004

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PATENT
Attorney Docket No.: 015280-317100US
Client Ref. No.: DHHS Ref. No.: E-059-97/1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

FitzGerald et al.

Application No.: 09/381,497

Filed: February 17, 2000

For: RECOMBINANT ANTIBODIES AND IMMUNOCONJUGATES TARGETED TO CD-22 BEARING CELLS AND TUMORS

Customer No.: 20350

Confirmation No. 4036

Examiner: Larry R. Helms, Ph.D.

Technology Center/Art Unit: 1642

SUPPLEMENTAL RESPONSE

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Supplemental to Applicants' Amendment mailed for filing on February 20, 2004, Applicants respectfully request entry of the Rule 1.132 Declaration of Dr. David J. Fitzgerald submitted herewith.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned.

Respectfully submitted,

Jean M. Lockyer, Ph.D.
Reg. No. 44,879

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PATENT
Attorney Docket No.: 015280-317100US
Client Reference No.: E-059-97/1f

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

FitzGerald *et al.*

Application No.: 09/381,497

Filed: September 20, 1999

For: RECOMBINANT ANTIBODIES AND IMMUNOCONJUGATES TARGETED TO CD-22 BEARING CELLS AND TUMORS

Examiner: Larry R. Helms, Ph.D.

Art Unit: 1642

DECLARATION OF DR. DAVID J. FITZGERALD UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Dr. David J. FitzGerald, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. I received a Ph.D. in Microbiology in 1982 from the University of Cincinnati, College of Medicine, in Cincinnati Ohio.

2. I am currently employed as the Chief of the Biotherapy Section, Laboratory of Molecular Biology in the Division of Basic Science of the National Cancer Institute at the National Institutes of Health where I conduct research relating to immunotoxins. I have authored

over 170 peer-reviewed scientific publications and chapters in this area. A copy of my curriculum vitae was previously submitted with Applicants' response filed May 2, 2001.

3. I have read and am familiar with the contents of the application. The claims currently at issue are drawn to a recombinant immunoconjugate that comprises a disulfide-stabilized RFB4 binding fragment linked to a therapeutic moiety. I understand that the Examiner has rejected the claims based upon his belief that the claimed recombinant immunoconjugates are obvious over the prior art. In particular, the Examiner alleges that the sequences of the RFB4 heavy and light chains were obvious in view of the existence of the known RFB4-producing hybridoma and techniques to obtain the V_H and V_L nucleic acid sequences. Further, he argues it would have been obvious to use these nucleic acid sequences to produce the dsFv-containing immunoconjugates in view of art describing the construction of dsFv antibodies. In this Declaration, I will present evidence that RFB4-containing immunoconjugates have superior expression characteristics and stability in comparison to a recombinant anti-CD22 immunoconjugate containing a different anti-CD22 antibody. Furthermore, this in this Declaration, I attest to the surprising binding characteristics and cytotoxicity of the claimed immunotoxin.

4. RFB4 immunoconjugates are generated by recombinant technology. Thus, the RFB4 component must express well. As one of skill in the art, the RFB4 V_H and V_L sequences are expressed surprisingly well and recombinant conjugates generated using them exhibit superior binding properties. In contrast, we have previously attempted to construct another recombinant anti-CD22 immunoconjugate using sequences from a different antibody, LL2. The LL2 V_H and V_L regions were very difficult to express and moreover, recombinant LL2-PE38 immunoconjugate exhibited poor cytotoxicity.

5. We first attempted to construct a single chain (sc) LL2 binding fragment. The genes encoding the V_H and V_L variable domains were obtained by PCR using primers to the known sequence. Restriction sites for assembling the peptide linker sequence, $(Gly_4Ser)_3$, that connects the V_H and V_L domains and for cloning into the expression vector were also introduced

by PCR. An expression vector was created that contained a CD22 V_H-linkerV_L-PE38 fusion construct. The expression plasmids were expressed in *E. coli* BL21 (λdE3). The yield of immunotoxin obtained was very low. Moreover, cytotoxicity of the small amount of immunotoxin that was obtained was very poor.

6. Cytotoxicity was evaluated using CA46 and Daudi Burkitt's lymphoma cells. The IC_{50} value, the concentration of immunotoxin that caused a 50% inhibition of protein synthesis, was determined after a 20-hour incubation with the toxin. The scLL2-PE38 immunoconjugate showed an IC_{50} of 1μg/ml for both CA46 and Daudi cells. Attempts were made to increase both the yield of the expressed product and the cytotoxicity of the immunotoxin. These changes resulted in only a slight improvement in expression. Cytotoxicity of this conjugate was also somewhat improved, but still exhibited an IC_{50} of only about 250 ng/ml. Our attempts to produce a recombinant ds(Fv) LL2 immunotoxin also failed due to the poor expression characteristic of the individual variable chains.

7. In contrast, RFB4 immunotoxin is expressed at much higher levels, is stable, and has superior binding characteristics and superior toxicity. These properties are unpredictable. First, it is acknowledged in the art that unknown parameters influence the degree of expression of the variable chain regions of different antibodies. Such parameters include the epitope that an antibody binds, and the folding properties of the recombinant antibodies. The art cannot predict which antibody sequence will express well or be stable, and hence, which immunotoxins can be produced at high levels.

8. Second, RFB4 immunotoxin, *e.g.*, RFB4ds(FV)-PE38, not only expresses well, but also retains the binding specificity and affinity of RFB4 IgG. This is unusual and surprising, not only in contrast to LL2-containing immunoconjugates, but in comparison to many recombinant immunotoxins. Typically, binding affinity is lowered in comparison to the parent antibody.

9. Last, the toxicity of the recombinant RFB4ds(FV)-PE38 was over 100 times better than any immunotoxin that could be produced using LL2 as the binding moiety. Further, this immunotoxin showed potent antitumor activity not only in animal models, but also in human Phase I trials, as described in my previous Declaration, already of record, signed May 15, 2001.

10. In summary, the high level of expression, retention of parental IgG binding affinity, and superior toxicity and efficacy of RFB4ds(FV) -PE38 is surprising and cannot be predicted from the art.

11. All statements herein made of my own knowledge are true and statements made on information or belief are believed to be true. The experimental work described herein was either conducted by myself or by a co-inventor, Dr. Ira Pastan or Dr. Robert Krietman, or under our direction.

Dated: March 11th 2004

David J. FitzGerald, Ph.D.